# Membrane Lipids and Proteins Involved in Plant Immunity

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The plasma membrane (PM), which is composed of a lipid layer implanted with proteins, has diverse functions in plant responses to environmental triggers. The heterogenous dynamics of lipids and proteins in the plasma membrane play important roles in regulating cellular activities with an intricate pathway that orchestrates reception, signal transduction and appropriate response in the plant immune system. In the process of the plasma membrane participating in defense responses, the cytoskeletal elements have important functions in a variety of ways, including regulation of protein and lipid dynamics as well as vesicle trafficking.

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## 1. Introduction

In order to survive and reproduce, plants have evolved two efficient immune systems. When plants are attacked by pathogens, plants use pattern recognition receptors (PRRs) on the surface of the cytoplasmic membrane to recognize microbial/pathogen-associated molecular patterns (MAMPs/PAMPs) or host-derived damage-associated molecular patterns (DAMPs) to further activate the first immune system called pattern-triggered immunity (PTI) [1][2][3]. For successful infection and colonization, pathogens secrete effectors into plant cells that interfere with host physiology and inhibit PTI [4]. The second layer of immune barrier activation is related to disease intracellular immune receptors, which belong to the nucleotide-binding leucine-rich repeat (NLRs) class and trigger immunity by identifying effector factors. Therefore, the mechanism of effector–host recognition is named effector-triggered immunity (ETI) [4]. Although PTI and ETI are involved in the activation of two unique types of receptors, the downstream immune outputs are strikingly similar [5][6]. It should be noted that these overlapping immune outputs include changes in $Ca^{2+}$ flux, rapid bursts of reactive oxygen species (ROS), mitogen-activated protein kinase (MAPK) cascades, transcriptional reprogramming and phytohormone signaling, indicating the junction and convergence of these two signaling cascades [7]. Furthermore, ETI is not a separate immune pathway which relies on the PTI machinery to function effectively [8]. Roux et al. [9] reported that the co-receptors BAK1 and BKK1 in PTI are also required for ETI response against *Hyaloperonospora arabidopsidis* (*Hpa*), suggesting PTI and ETI share central components of these two systems; the two immune responses combine to promote a strong immunity. Though growing evidence points to the presence of complex interactions between PRR- and NLR-mediated signaling cascades [10], their connection is unknown.

Plant immunity is closely associated with the cell cytoskeleton. The plant cytoskeleton is composed of microfilaments (MFs) and microtubules (MTs). MFs, also known as the actin cytoskeleton, are generated by

polymerizing globular (G)-actin into filamentous (F)-actin [11]. MTs are made up of a complicated array of α/β-tubulin heterodimers [12]. The cytoskeleton plays an important role in the process of disease resistance, especially the actin cytoskeleton, which is a signal transduction platform in the plant immune process. The increase in actin filament density is one of the conserved PTI responses [13]. After MAMP or DAMP treatment, the actin filament density increased significantly at the infection site [14]. Moreover, fungal invasion of plants triggers MF and MT reorganization, leading actin filaments to form radial microfilament bundles, which are conducive to the transport of organelles and vesicles to the invasion site and enhance the resistance of pathogenic fungal invasion [15]. When the host plant is infected by powdery mildew, host nucleus, ER and Golgi accumulation occur concurrently with fast reorganization of actin filaments [15]. The use of actin polymerization inhibitor latrunculin B (Lat B) to inhibit the actin filaments aggregation affects penetration resistance, causing plants to increase the susceptibility of pathogenic bacteria [13]. In addition, the cytoskeleton regulates callose deposition in the PTI immune response. Yang et al. [15] have reported that inactivation of myosin by pharmacological inhibitors prevents deposition of callose into the apoplastic papillae, resulting in the attenuation of penetration resistance. The translocation of callose synthases to the PM requires both actin filaments and MT, and the disruption of either cytoskeletal network causes callose synthases to dysfunction [16]. The cytoskeleton plays a positive regulatory role in plant immunity, actively responding to pathogenic signals and rapidly changing its arrangement to resist pathogenic infection.

# 2. Membrane Lipids and Proteins Involved in Plant Immunity

The plasma membrane provides a natural barrier for the cell, which is composed of lipids and proteins. Almost all the important functions of the cell are related to the plasma membrane, which not only protects the cell but also participates in many physiological processes such as cell signal recognition and transmission, material transportation, etc. [10]. The plasma membrane plays a key role during pathogen attack. It is involved in pathogen recognition and the transmission of external signals into the downstream of the cell, which further helps to regulate plant immunity responses.

## 2.1. Functional Diversity of the Membrane Lipids That Are Involved in Immunity Response

Membrane lipids include phospholipids and sterols in higher plants, and the constituents play a critical role in maintaining the stability of the cell membrane structure and function. Phospholipids, which consist of glycerophospholipids and sphingolipids, are abundant in all plasma membranes. Glycerophospholipids are widely conserved in animals and plants, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and diacylglycerols (DAGs). Phospholipid-based signal transduction has been reported to be involved in plant immune responses. For example, phospholipase D can be hydrolyzed to generate phosphatidic acid, which serves the function of second messenger to activate ROS to trigger actin rearrangements [14].

Sphingolipids, which account for up to 40% of total lipids in plants [17], can be grouped into four classes in plants: free long-chain bases (LCBs); ceramides (CERs), comprising LCBs with fatty acids (FA); glucosylceramides; and

glycosyl inositol phosphoryl ceramides (GIPCs) [18]. The 2-hydroxylation of acyl chains in plant sphingolipids are a typical hydroxylation modification, which are important in stress responses. A recent study showed that 2-hydroxy sphingolipids contribute to the formation of plasma membrane lipid rafts [19]. The formation of plasma membrane domains also requires the aggregation of sterols. It has been identified that plants sterols contain around 250 different types of sterols and sterol conjugates, including free sterols, sterol esters, sterol glycosides and acyl sterol glycosides [20]. Particularly, sterol glycosides and acyl sterol glycosides gather in lipid rafts [21].

The lipid raft/membrane raft is a dynamic domain of a liquid-ordered phase with a diameter of 10 to 200 nm, which is characterized by tight lipid filling [17]. It is reported that these regions are resistant to extraction by non-ionic detergents at low temperatures, so they are also known as detergent-resistant membranes (DRMs) [22]. A variety of physiological and biochemical reactions in cells take place on membrane raft microdomains, which are considered as the platform that recruit multiple immune molecules to participate in the defense response. Moreover, Cui et al. [23] reported that flagellin sensing2 (FLS2) protein was localized to the microdomain through oligomerization after being stimulated by flg22. A similar pattern of results was obtained in the study of Remorin1.3 (REM1.3) [24].

## 2.2. Functional Diversity of the Membrane Proteins That Are Involved in Immunity Response

Membrane proteins are important executors of plasma membrane function. When plants are attacked by pathogens, plants employ PRRs to recognize specific chemical components of invading pathogenic microorganisms and activate the plant immune system to make an immune response. Most PRRs contain an extracellular domain with leucine-rich repeats (LRRs), an intracellular kinase domain and a single-pass transmembrane domain. At present, the well-studied PRRs include FLS2, elongation factor Tu receptor (EFR), chitin elicitor receptor kinase 1 (CERK1) and PEP Receptors (PEPRs) [25]. PRR is the immune switch of PTI, and the first PRR identified in plants was *Arabidopsis* FLS2. After the extracellular LRR domain of FLS2 recognizes bacterial flagellin, FLS2 interacts with the co-receptor BAK1 to form a stable heterodimer [26][27]. Similar to FLS2, the EFR recognizes the EF-Tu and elf18 peptides and forms EFR–BAK1 complexes to activate plant immunity [28]. The perception of PEP by a plant elicitor peptide receptor (PEPR) leads to the fluctuation of $Ca^{2+}$ to trigger the downstream immune signal [29]. Importantly, PRRs can also bind effectors, including FLS2 and EFR, to hinder immune responses. For example, FLS2 can directly interact with *Pseudomonas syringae* effector AvrPto to inhibit the kinase activity of FLS2 [30].

In addition, NLRs on the plasma membrane have been reported to recognize effectors, which are secreted by pathogens, directly or indirectly. Plant NLRs contain three domains: an N-terminal variable domain, a middle nucleotide binding domain and a C-terminal LRR domain [31]. NLRs are classified into two groups that are defined by different N-terminal domains: the coiled-coil-type NLRs (CNLs) and Toll/interleukin-1 receptor/resistance protein type NLRs (TNLs) [32]. After immune activation, NLRs often form oligomeric complexes, which are called resistosomes in plants. For instance, CNL receptor hopz-activated resistance 1 (ZAR1) in *Arabidopsis* indirectly recognizes the effector AvrAC through bait protein PBL2. AvrAC uridylylates the PBL2, which is then recruited to form a ZAR1–RKS1–$PBL2^{UMP}$ complex [33]. Further work proved that ZAR1-activated resistosome serves as a

calcium-permeable cation channel to initiate immunity response, such as production of ROS and cell death [34]. Another typical representative of TNL resistance protein is *Arabidopsis thaliana* recognition of peronospora parasitica1 (RPP1). RPP1 directly combines with *Hpa* effector ATR1 to form tetrameric resistosome, which promotes the NADase hydrolytic activity to activate downstream EDS1 and NRG1 immune pathways [35][36].

Besides receptors, other membrane proteins have also been reported to participate in the defense response, such as respiratory burst homologs (RBOHs) and plasma membrane intrinsic proteins (PIPs). ROS produced via RBOH appear to cause local and/or systemic reprogramming in order to activate innate immunity [37]. Studies about leaf microbiota show that RBOHD and RBOHF maintain the homeostasis of the microbiota to prevent imbalances [38]. PIPs not only transport water but also extend to subcellular transport of ROS, such as $H_2O_2$ [39]. Studies have shown that AtPIP2;1 was phosphorylated by BAK1 during the response of guard cells to flg22 and increased the transport rate of water and $H_2O_2$, thereby regulating the closure of stomata to achieve an immune response [40]. Interestingly, due to the three extracellular regions of PIPs that are exposed to the extracellular environment, plant pathogenic bacteria will take the opportunity to hijack certain PIPs to promote infection and play a role in causing disease and increasing virulence. OsPIP1;3 interactions with the bacterial hydrophilic protein *Hpa1* may result in conformational changes of the host cell membrane and contribute to the transport of effector proteins into host cells [41][42].

Furthermore, some proteins do not directly participate in the defense response but form specific microdomains with membrane lipids, thus recruiting receptors to participate in the defense response. Overexpression of NbHIR3.2 or OsHIR3 increased resistance to *Pseudomonas syringae pv*. *tomato* strain DC3000 (*Pst*DC3000) and *Xanthomonas* in tobacco and rice, respectively [43]. OsHIR1 can interact with rice Leucine-Rich Repeat protein 1 (OsLRR1) during pathogen invasion to trigger hypersensitive cell death [44]. Remorins, which are the best characterized marker of plasma membrane microdomains in plants [45], play key roles in plant immunity. For instance, ZmREM6.3 was first reported to be associated with fungal interactions and quantitative disease resistance [46]. In addition, AtREM1.2 interacts with a regulator of PTI and ETI, AtRIN4, indicating that AtREM1.2 can recruit AtRIN4 into membrane microdomains to regulate basal defense [47]. New research proposed that upon perception of PAMPs, Remorin undergoes assembly via intrinsically disordered region mediated oligomerization and recruits Formin into membrane microdomains, which in turn increases actin nucleation [48].

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